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PLANT GROWTH-PROMOTING BACTERIA AND GENERATIVE AI VALIDATION IN SUSTAINABLE LETTUCE SEEDLING PRODUCTION SYSTEMS

BACTÉRIAS PROMOTORAS DO CRESCIMENTO DE PLANTAS E VALIDAÇÃO DE IA GENERATIVA EM SISTEMAS SUSTENTÁVEIS DE PRODUÇÃO DE MUDAS DE ALFACE

BACTERIAS PROMOTORAS DEL CRECIMIENTO VEGETAL Y VALIDACIÓN DE IA GENERATIVA EN SISTEMAS SOSTENIBLES DE PRODUCCIÓN DE PLÁNTULAS DE LECHUGA

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ABSTRACT

The production of lettuce seedlings (Lactuca sativa L.) is a fundamental process for maintaining the good quality of the final product, as well as for maintaining the sustainability of crops. The objective of this study was to evaluate the potential of nine bacterial isolates to promote the growth of lettuce seedlings. To this end, two production cycles were conducted using 120-cell trays and inert substrate fertilized with mineral fertilizers in a greenhouse at Embrapa Hortaliças, Federal District, Brazil. A completely randomized design (CRD) was used with five replicates and 12 treatments, including three controls (use of mineral phosphorus, no use of mineral phosphorus, and use of a commercial P-solubilizing biofertilizer). The other nine treatments consisted of bacterial inoculants with phosphate solubilization potential and siderophore production previously tested *in vitro*. In these, no mineral phosphorus was added during fertilization. The following variables were evaluated: average number of leaves, average leaf width, aerial part length, and root length. The data were submitted to analysis of variance and the means were grouped by the Scott-Knott test. The quantitative consolidation of the results of the

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two production cycles was done by calculating the average rank. Visual analysis was also conducted by human experts (two PhDs) and Generative Artificial Intelligence (AIs - LLMs). For this purpose, ChatGPT5, Gemini, and Copilot were tested. The analysis was performed using Prompt Engineering and Prompt Chaining proposed in this work and photos attached to the AIs. Only ChatGPT and Gemini showed good results, compatible with human observation (appropriate Spearman's Correlation Coefficient and Mean Deviation Average). The consolidation of the rankings based on quantitative data and visual analysis by the AIs and human observation identified T7, T5, and T8 as the best treatments, with T7 standing out. This work is in line with the current needs of sustainable, resilient, and adapted agriculture.

Keywords: Sustainable Agriculture. Resilient and Adapted Agriculture. Low-Carbon Agriculture. Bio-Inputs. Biofertilizers. Artificial Intelligence in Agriculture.

RESUMO

A produção de mudas de alface (Lactuca sativa L.) é um processo fundamental para manutenção de boa qualidade do produto final, bem como para manutenção da sustentabilidade dos cultivos. O presente estudo teve como objetivo avaliar o potencial de nove isolados bacterianos com relação ao seu potencial de promoção de crescimento de mudas de alface. Para tal, dois ciclos produtivos foram conduzidos utilizando-se bandejas de 120 células e substrato inerte fertilizado com adubos minerais, em casa de vegetação na Embrapa Hortaliças, Distrito Federal, Brasil. Utilizou-se o delineamento inteiramente casualizado (DIC) com cinco repetições e 12 tratamentos, sendo três controles (utilização de fósforo mineral, sem utilização de fósforo mineral e utilização de um biofertilizante solubilizador de P comercial). Os outros nove tratamentos consistiam em inóculos bacterianos com potencial de solubilização de fosfato e produção de sideróforos testados *in vitro* previamente. Nestes, não houve adição de fósforo mineral durante a fertilização. Foram avaliadas as seguintes variáveis: número médio de folhas, largura foliar média, comprimento da parte aérea e comprimento radicular. Os dados foram submetidos à análise de variância e as médias agrupadas pelo teste de Scott-Knott. A consolidação quantitativa dos resultados dos dois ciclos produtivos se deu por meio do cálculo do rank médio. Foi também conduzida a análise visual por meio de especialistas humanos (dois Phd) e Inteligências Artificiais Generativas (IAs - LLMs). Para tal, foram testadas: ChatGPT5, Gemini e Copilot. A análise foi efetuada a partir de Engenharia de Prompt e Prompt Chaining propostos nesse trabalho e de fotos anexadas às IAs. Apenas ChatGPT e Gemini apresentaram bons resultados, compatíveis com a observação humana (Coeficiente de Correlação de Spearman e Mean Deviation Average adequados). A consolidação dos ranqueamentos efetuados a partir dos dados quantitativos e da análise visual pelas IAs e da observação humana identificou T7, T5 e T8 como melhores tratamentos, com destaque para T7. O presente trabalho alinha-se às necessidades atuais da agricultura sustentável, resiliente e adaptada.

Palavras-chave: Agricultura Sustentável. Agricultura Resiliente e Adaptada. Agricultura de Baixa Emissão de Carbono. Bioinsumos. Biofertilizantes. Inteligência Artificial na Agricultura.

RESUMEN

La producción de plántulas de lechuga (Lactuca sativa L.) es fundamental para mantener la calidad del producto final y la sostenibilidad de los cultivos. El objetivo de este estudio fue evaluar el potencial de nueve aislados bacterianos para promover el crecimiento de plántulas de lechuga. Para ello, se realizaron dos ciclos de producción utilizando bandejas de 120 celdas y sustrato inerte fertilizado con fertilizantes minerales en un invernadero de Embrapa Hortaliças, Distrito Federal, Brasil. Se utilizó un diseño completamente aleatorizado (DCA) con cinco réplicas y 12 tratamientos, incluyendo tres controles (uso de fósforo mineral, ausencia de fósforo mineral y uso de un biofertilizante comercial solubilizante de fósforo). Los otros nueve tratamientos consistieron en inóculos bacterianos con potencial de solubilización de fosfato y producción de sideróforos, previamente probados in vitro. En estos, no se añadió fósforo mineral durante la fertilización. Se evaluaron las siguientes variables: número promedio de hojas, ancho promedio de las hojas, longitud de la parte aérea y longitud de la raíz. Los datos se sometieron a análisis de varianza y las medias se agruparon mediante la prueba de Scott-Knott. La consolidación cuantitativa de los resultados de los dos ciclos de producción se realizó mediante el cálculo del rango promedio. El análisis visual también fue realizado por expertos humanos (dos doctores) e Inteligencia Artificial Generativa (IA - LLM). Para ello, se probaron ChatGPT5, Gemini y Copilot. El análisis se realizó utilizando la Ingeniería de Prompt y el Encadenamiento de Prompt propuestos en este trabajo, junto con fotos adjuntas a las IA. Solo ChatGPT y Gemini mostraron buenos resultados, compatibles con la observación humana (coeficiente de correlación de Spearman y promedio de desviación media adecuados). La consolidación de las clasificaciones, basada en datos cuantitativos y el análisis visual realizado por las IA y la observación humana, identificó a T7, T5 y T8 como los mejores tratamientos, destacando T7. Este trabajo se ajusta a las necesidades actuales de una agricultura sostenible, resiliente y adaptada.

Palabras clave: Agricultura Sostenible. Agricultura Resiliente y Adaptada. Agricultura Baja en Carbono. Bioinsumos. Biofertilizantes. Inteligencia Artificial en la Agricultura.

INTRODUÇÃO

Lettuce (*Lactuca sativa* L.) is one of the most widely grown and consumed leafy vegetables in Brazil and worldwide, notable for its short cycle, high nutritional value, and broad market acceptance (Shi et al., 2022; Zappeline et al., 2024). The production of quality seedlings is essential for the success of other stages of the production cycle, directly influencing initial establishment, plant stand uniformity, plant health, and, consequently, productivity and nutritional quality (Souza et al, 2019; Oliveira et al., 2024).

Normally, lettuce seedling production depends on the use of commercial substrates enriched with mineral fertilizers or even fertilized on site with the latter. However, these inputs are costly and promote dependence on external inputs, in addition to potentially generating negative environmental impacts (Nurhayati et al., 2024). All these factors have led to a growing search for sustainable alternatives for agricultural production, to make it more resilient. Recently, strategies have been developed to increase crop sustainability, such as the use of biochar derived from agro-industrial waste, alternative substrates, microalgae biofertilizers, among others. These practices can promote development similar to commercial substrates, contributing to sustainability, reducing costs, and the environmental footprint (Alvarez-González et al., 2025). There is a recent global trend towards seeking alternatives that diversify agricultural inputs, promoting sustainability and resilience, and reducing dependence on synthetic inputs (Ré et al., 2024).

Although the use of bio-inputs such as biofertilizers, for example, are important alternatives in the pursuit of sustainability, they still present challenges such as the heterogeneity of their compositions, which hinders the standardization of plant production (Cajamarca et al., 2019). Although the use of bio-inputs such as biofertilizersare important alternatives in the pursuit of sustainability, they still present challenges such as the heterogeneity of their compositions, which hinders the standardization of plant production (Cajamarca et al., 2019). In this sense, the production of bio-inputs with specific functions, compositions, and concentrations using plant growth-promoting microorganisms (PGPMs) stands out as a promising biotechnological tool. These microorganisms include bacteria, actinomycetes, fungi, yeasts, among others, which act in biological nutrient fixation (BNF), phosphate solubilization, and the production of siderophores and phytohormones such as auxin (AIA) (Bomfim et al., 2024). These characteristics make PGPMs not only alternatives for providing nutrients to seedlings and plants, but also inducers of tolerance and resistance to biotic and abiotic stresses (Junrami *et al*., 2022; Tang *et al*., 2022; Anand *et al*., 2023). This fact is especially important in the current scenario of global climate change (GCCs) (Lima et al., 2024; Lima et al., 2025).

This study is a continuation of the one conducted by Bomfim et al. (2024). These authors showed that 217 isolates of microorganisms were isolated from a biofertilizer called Hortbio. The most common bacteria phyla determined were Firmicutes (44%), Proteobacteria (41%), Actinomycetes (13%) and Bacteroidetes (2%). Yeasts, in turn, presented the phylum Ascomycota as the most prevalent, with a predominance of the genera Candida and Pichia. Another phylum identified was Basidiomycota (genus Rhodotorula). Finally, about filamentous fungi, isolates of the genera Galactomyces, Penicillium, Mucor, Aspergillus, Cladosporum, and Trichoderma were found. Consultation of genomic databases revealed the existence of isolates with potential for FBN, phosphate solubilization, and siderophore production, biological control, auxin production, organic waste decomposition, among others.

For all the above reasons, the objective of this study was to evaluate the potential of nine bacterial isolates in promoting the development of lettuce seedlings.

MATERIAL AND METHODS

Location of the Study Area

The study was conducted at the Embrapa Vegetables experimental field, in a protected environment (greenhouse), located at the geographical coordinates 15°55'54.83“S and 48° 8'42.60”W. The local climate is tropical savanna (Aw - Köppen-Geiger system).

Seedling Production

The seedlings were produced by sowing curly lettuce seeds in 120-cell trays filled with an inert substrate without phosphate fertilization. They were watered manually twice a day.

Production of Formulations Containing Microbial Isolates

The following microbial isolates were used to conduct this study: B10.02; B10.05; B10.06; B10.11; B10.13; B10.14; B10.17; B10.19; B10.20. The letter B refers to the bacterium, while the numerical code refers to the registration code in our microorganism database. For intellectual property reasons, since the products are intended to be commercially registered, it is not possible to disclose the phylum, genus, or species to which the isolates belong. However, all microorganisms used showed good phosphate solubilization capacity and siderophore production *in vitro* tests. The microbial isolates were produced in sucrose medium, at a concentration of 10^8 UFC/mL, with Erlenmeyer flasks being shaken orbitally for 12 hours. The cultures were centrifugated and resuspended in 0.85% saline solution.

Experimental Design

A completely randomized design (CRD) was used, where each tray of 120 cells constituted a treatment. Twelve treatments were used, namely: T1 - Conventional seedling production with P fertilization; T2 - Seedling production without P fertilization; T3 - Seedling production without P fertilization and with application of a commercial biofertilizer; T4 - inoculum B10.02; T5 - inoculum B10.05; T6 - inoculum B10.06; inoculum B10.11; T8 - inoculum B10.13; T9 - inoculum B10.14; T10 - inoculum B10.17; T11 - inoculum B10.19; and T12 - inoculum B10.20. Five replicates were used. All bacterial inoculants showed potential for phosphate solubilization and siderophore production *in vitro* experiments.

Morphophysiological Parameters Used

The following morphophysiological parameters were evaluated in this study: average number of leaves (ANL); average leaf width (ALW); average shoot length (ASL); root length (RL).

Statistical Analysis

The data were submitted to the Shapiro-Wilk normality test, Analysis of Variance, and the means were tested and grouped by Scott-Knott. Descriptive statistical analyses such as the determination of means, standard deviation, and 95% confidence interval were also performed. ANOVA and mean tests were conducted using SISVAR software.

Use of Generative Artificial Intelligence (LLMs)

Artificial intelligence (AI) was used to evaluate certain parameters, such as the visual quality of the seedlings produced. For this task, three IAs were used as analysis support. The same prompts were used as those applied to the three AIs: ChatGPT - OpenAI, Microsoft Copilot, and Gemini - Google. All results obtained underwent human screening (PhD), having been filtered and associated with the observations made *in situ*.

AIs was also used to prepare figures and graphs, as well as to calculate the average rank, which aimed to define the best treatments. All these steps were performed using Prompt Engineering and Chaining in AI Data Analyst (Open AI). These digital tools were also used to assist with spell checking and translation into English (DeepL Write, ChatGPT – OpenAI, Microsoft Copilot and Gemini - Google). All uses were performed under the supervision of human experts in the field, with a minimum of a PhD.

Prompt Engineering and Prompt Chaining Used as Support for Visual Analysis

The following Prompt Engineering and Prompt Chaining were used to improve the performance of the AIs used:

For first cycle

Prompt 1 - Take on the persona of an Agricultural Engineer with a PhD in agricultural microbiology and at least 15 years of experience in developing and testing bio-inputs and their effects on vegetable production and physiology.

Prompt 2 - Please take note of and carefully analyze the patterns in the first productive cycle attached photo.

Prompt 3 - Keep in mind that both photos refer to an experiment that evaluated the performance of different microbial inoculants that solubilize phosphorus, produce siderophores, and promote plant growth on the production of lettuce seedlings.

Prompt 4 - Analyze the following patterns in both photos: intensity of green coloration, uniformity of seedling stands, presence of signs of physiological disorders commonly occurring in lettuce, such as chlorosis or edge burning, for example.

Prompt 5 - Rank the treatments from best to worst based on the observations made earlier.

For second cycle

Prompt 1 - Take on the persona of an Agricultural Engineer with a PhD in agricultural microbiology and at least 15 years of experience in developing and testing bio-inputs and their effects on vegetable production and physiology.

Prompt 2 - Please take note of and carefully analyze the patterns in the second productive cycle attached photo.

Prompt 3 - Keep in mind that both photos refer to an experiment that evaluated the performance of different microbial inoculants that solubilize phosphorus, produce siderophores, and promote plant growth on the production of lettuce seedlings.

Prompt 4 - Analyze the following patterns in both photos: intensity of green coloration, uniformity of seedling stands, presence of signs of physiological disorders commonly occurring in lettuce, such as chlorosis or edge burning, for example.

Prompt 5 - Rank the treatments from best to worst based on the observations made earlier.

Integrated Prompt - Now combine visual analysis with quantitative average ranking for both cycles integrated to determine the best treatments. Only consider treatments that showed at least uniformity in the seedling stand as good treatments.

Integration of Visual Rankings from the Two Production Cycles for Each Evaluation Method, Validation and Integration Consistency of IA Uses as "Evaluator"

For each "evaluator" (human, ChatGPT, Gemini, and Copilot), the rankings obtained per cycle were integrated into a single index, which compiles the visual results obtained in each of the two production cycles. The integration was performed individually, by "evaluator," following a standardized methodology based on ranking normalization and average aggregation, as described below:

Then, each of the two rankings (referring to cycles 1 and 2) for each "evaluator" was converted into normalized values between 0 and 1, using the position of each ranking for each treatment as a basis, following the equation:

$$N_{iC} = 1 - \frac{(r_{iC} - 1)}{(k - 1)}$$

Equation 1. Calculation of normalized rank scores.

Where:

N_{iC}= normalized rank score of treatment *i* in cycle *C* (1 or 2);
r_{iC}= raw visual rank position of treatment *i* in cycle *C*;
k= total number of treatments (12).

The next step was to integrate the results for each of the production cycles defined by each "evaluator." To do this, equal weights (50%) were used for results for each one. This generated an integrated visual score. The following equation was used for this purpose:

$$R_i^{(E)} = \frac{N_{i1} + N_{i2}}{2}$$

Equation 2. Calculation of integrated scores for each treatment.

Where:

$R_i^{(E)}$= integrated score of treatment i for evaluator E (Human, ChatGPT, Gemini or Copilot);
N_{i1} and N_{i2}= normalized scores from cycles 1 and 2.

For ranking and tie-breaking rules, treatments were placed in descending order of $R_i^{(E)}$. When the difference between values was less than or equal to 2% (arbitrarily defined number), they were considered equal. The final ranking was then used for cross-comparison and to compare the performance of each AI relative to human observation.

The consistency of the integrated rankings, resulting from the application of Prompt Engineering and the proposed Prompt Chaining, for each AI, was then evaluated using Spearman's correlation (ρ) and Mean Absolute Deviation (MAD) as tools. Both analyses are performed using Equations 3 and 4.

$$\rho_E = 1 - \frac{6\sum d_i^2}{n(n^2-1)}$$

Equation 3. Calculation of Spearman's correlation coefficient.

$$MAD_E = \frac{1}{n}\sum |r_{i,mean} - r_{i,I}|$$

Equation 4. Calculation of Mean Absolute Deviation (MAD).

Where:

- d_i= difference between integrated and cycle-specific positions;
- $r_{i,mean}$= mean rank position across cycles;
- $r_{i,I}$= final integrated position.

Observation: $\rho \geq 0.85$ and MAD ≤ 1.5 were considered evidence of strong correlation and stable integration across cycles.

RESULTS AND DISCUSSION

Results

ANOVA and Scott-Knott Test for Morphophysiological Parameters

All data analyzed showed normal behavior. The results of the analysis of variance are shown in Table 1.

Table 1

Summary of the analysis of variance of the morphophysiological parameters evaluated: average number of leaves (ANL); average leaf width (ALW); average shoot length (ASL); root length (RL).

	Source of variation	ANL		ALW		ASL		RL	
First Cycle	**Input used**	QMR	Pr>Fc	QMR	Pr>Fc	QMR	Pr>Fc	QMR	Pr>Fc
		0.77	0.06	0.88	0.00	11.75	0.00	8.37	0.00
Second Cycle	**Input used**	QMR	Pr>Fc	QMR	Pr>Fc	QMR	Pr>Fc	QMR	Pr>Fc
		1.88	0.00	0.74	0.00	11.78	0.00	30.22	0.00

Table 2, in turn, shows the grouping of means by the Scott-Knott test. Means followed by the same letter are not statistically different. For ease of viewing, the results are also presented in graph format in Figures 1 to 4.

Table 2

Effect of microbial isolates on morphophysiological parameters of lettuce seedlings in two cycles: average number of leaves (ANL); average leaf width (ALW); average shoot length (ASL); root length (RL).

Source of variation	ANL	ALW	ASL	RL
		First Cycle		
T1	3.20^{ns}	2.38a	4.30d	7.40c
T2	3.20^{ns}	1.84b	4.80d	6.60c
T3	2.80^{ns}	1.66b	5.50c	6.60c
T4	3.40^{ns}	1.80b	8.40a	8.70b
T5	4.60^{ns}	2.30a	7.40b	10.0a
T6	3.40^{ns}	2.06b	6.10c	9.30a
T7	3.00^{ns}	2.60a	8.40a	7.50c
T8	3.20^{ns}	2.46a	6.12c	10.8a
T9	3.40^{ns}	2.10b	8.40a	7.70c

T10	4.80^{ns}	3.00a	6.30c	7.70c
T11	3.60^{ns}	2.46a	4.50d	7.80c
T12	3.40^{ns}	2.50a	5.20d	8.40c
Second Cycle				
T1	3.20b	2.70b	4.60b	7.70b
T2	5.40a	2.68b	3.40c	3.10c
T3	4.40b	2.38b	3.10c	3.60c
T4	5.40a	3.42a	3.10c	8.70b
T5	5.60a	3.20a	4.50b	8.90a
T6	5.20a	2.74b	3.60c	4.70c
T7	5.60a	3.38a	5.70a	8.80a
T8	5.00b	2.50b	5.20^{a}	10.70a
T9	5.40a	3.60a	4.30b	6.40b
T10	5.00b	2.68b	5.60a	7.30b
T11	4.80b	2.38b	5.20a	8.00b
T12	5.40a	2.92b	5.00a	9.30a

Averages followed by the same letter do not differ from each other at a 5% probability. Legend: T1 - Conventional seedling production with P fertilization; T2 - Seedling production without P fertilization; T3 - Seedling production without P fertilization and with application of a commercial biofertilizer; T4 - inoculum B10.02; T5 - inoculum B10.05; T6 - inoculum B10.06; inoculum B10.11; T8 - inoculum B10.13; T9 - inoculum B10.14; T10 - inoculum B10.17; T11 - inoculum B10.19; and T12 - inoculum B10.20. Five replicates were used. All bacterial inoculants showed potential for phosphate solubilization and siderophore production *in vitro* experiments.

The ANOVA of the first cycle showed a statistically significant difference for all parameters evaluated ($p \leq 0.05$), except for ANL. Even when the mean squares of the residuals (MSR) were low, Fc>P values were very low, indicating the robustness and consistency of the data obtained, increasing the reliability of the results (Walker, 2025). In the second cycle, this pattern is repeated, but with all parameters showing a statistically significant difference. These results point to significant effects caused by used bacterial bio-inputs such as PGPRs. For ANOVA to be robust, the residual error should be small. This provides a good explanation for the variability of the data, ensuring high reliability when there is a significant difference (Drummond & Vowler, 2012).

The fact that the second cultivation cycle presented ANOVA with statistically significant differences for all variables analyzed may suggest that continuous effects from the use of the tested bio-inputs are possible. This may occur due to several factors, such as the adaptation of microorganisms to the substrate and the cultivation environment. Although further investigation is needed, it is possible that the long-term use of the tested PGPRs may improve the agronomic effects on lettuce seedling production. The benefits of successive inoculation with PGPRs on morphophysiological parameters in seedling and agricultural production have been reported by Lyu et al. (2023). Benefits have also been observed for other vegetables in different production systems and environmental conditions, such as hydroponic lettuce (Ikiz et al., 2024) and tomato seedling production under water-scarce conditions (Astorga-Eló et al., 2021).

Figure 1

Effect of microbial isolates on morphophysiological parameters of lettuce seedlings in two cycles: average number of leaves (ANL). Legend: T1 - Conventional seedling production with P fertilization; T2 - Seedling production without P fertilization; T3 - Seedling production without P fertilization and with application of a commercial biofertilizer; T4 - inoculum B10.02; T5 - inoculum B10.05; T6 - inoculum B10.06; inoculum B10.11; T8 - inoculum B10.13; T9 - inoculum B10.14; T10 - inoculum B10.17; T11 - inoculum B10.19; and T12 - inoculum B10.20. Five replicates were used. All bacterial inoculants showed potential for phosphate solubilization and siderophore production in vitro experiments.

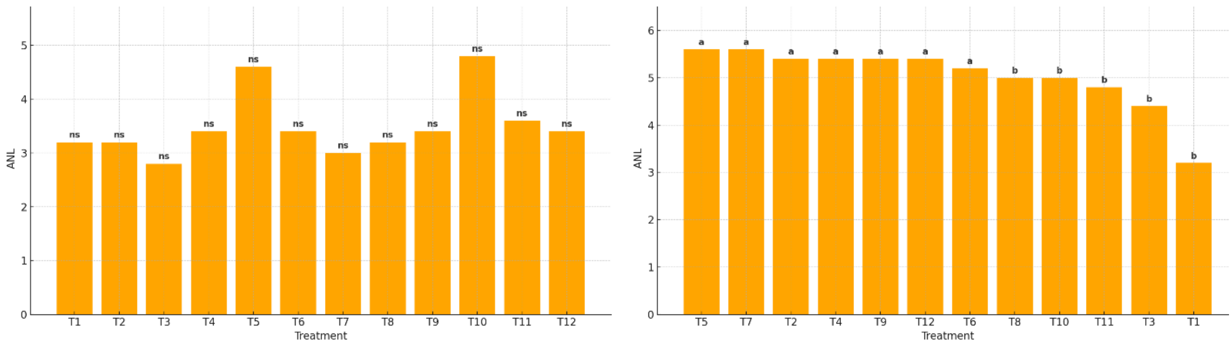
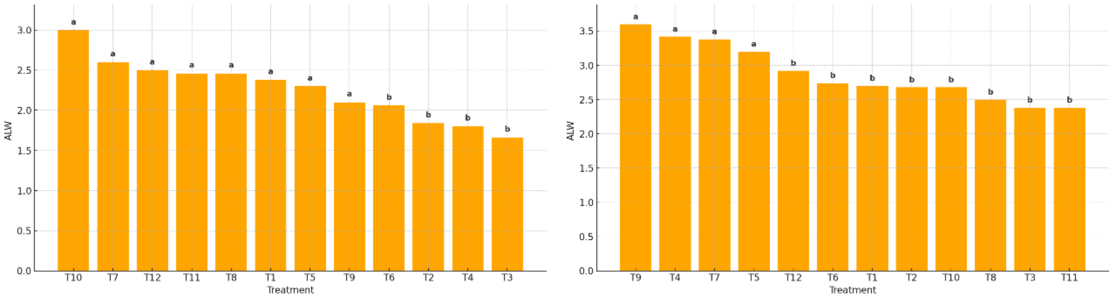


Figure 1 shows that, for the ANL variable, as previously discussed about the significance of ANOVA, there was no statistically significant difference measured by the Scott-Knott test for the first production cycle. In the second cycle, the test of means grouped treatments T5, T7, T2, T4, T9, T12, and T6 as those with the highest average number of leaves. The second and last group consisted of treatments T8, T10, T11, T3, and T1. The continued application of phosphorus-solubilizing and siderophore-producing PGPRs may have been responsible for the differences in the results observed in the two cycles.

Figure 2

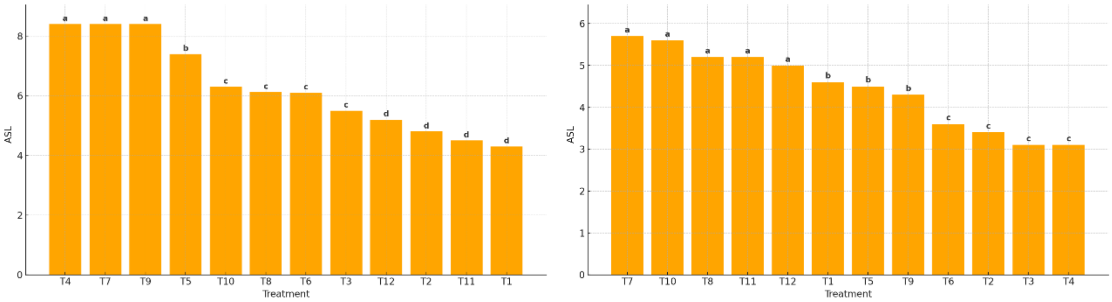
Effect of microbial isolates on morphophysiological parameters of lettuce seedlings in two cycles: average leaf width (ALW). Legend: T1 - Conventional seedling production with P fertilization; T2 - Seedling production without P fertilization; T3 - Seedling production without P fertilization and with application of a commercial biofertilizer; T4 - inoculum B10.02; T5 - inoculum B10.05; T6 - inoculum B10.06; inoculum B10.11; T8 - inoculum B10.13; T9 - inoculum B10.14; T10 - inoculum B10.17; T11 - inoculum B10.19; and T12 - inoculum B10.20. Five replicates were used. All bacterial inoculants showed potential for phosphate solubilization and siderophore production in vitro experiments.



Regard to average leaf width (ALW), in the first cycle, treatments T10, T7, T12, T11, T8, T1, T5 and T9 were more efficient, with the others that show poorer results. In the second cycle, only treatments T9, T4, T7 and T5 stood out, with the other treatments presenting lower ALW values

Figure 3

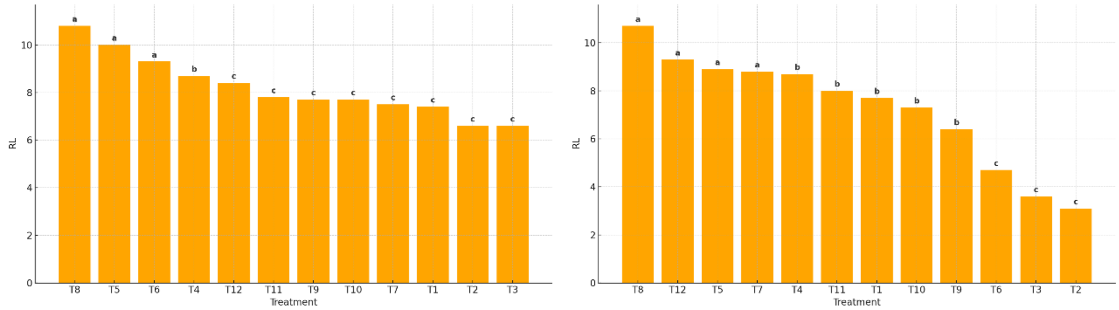
Effect of microbial isolates on morphophysiological parameters of lettuce seedlings in two cycles: average shoot length (ASL). Legend: T1 - Conventional seedling production with P fertilization; T2 - Seedling production without P fertilization; T3 - Seedling production without P fertilization and with application of a commercial biofertilizer; T4 - inoculum B10.02; T5 - inoculum B10.05; T6 - inoculum B10.06; inoculum B10.11; T8 - inoculum B10.13; T9 - inoculum B10.14; T10 - inoculum B10.17; T11 - inoculum B10.19; and T12 - inoculum B10.20. Five replicates were used. All bacterial inoculants showed potential for phosphate solubilization and siderophore production in vitro experiments.



Average Shoot Length (ASL) showed more heterogeneous behavior than ANL and ALW, demonstrating a more differentiated effect of bacterial compounds on this variable. In the first cycle, T4, T7, and T9 stood out, exceeding 8.3 cm. The second group consisted of treatment T5, with 7.4 cm. T10, T8, T6, and T3 made up the third group, while T12, T2, T11, and T1 made up the last group. In the second cycle, lower performance (lower ASL values) was observed in general. Even so, T7, T10, T8, T11, and T12 performed better. T1, T5, and T9 showed intermediate behavior. T6, T2, T3, and T4 showed the worst averages.

Figure 4

Effect of microbial isolates on morphophysiological parameters of lettuce seedlings in two cycles: average shoot length (ASL). Legend: T1 - Conventional seedling production with P fertilization; T2 - Seedling production without P fertilization; T3 - Seedling production without P fertilization and with application of a commercial biofertilizer; T4 - inoculum B10.02; T5 - inoculum B10.05; T6 - inoculum B10.06; inoculum B10.11; T8 - inoculum B10.13; T9 - inoculum B10.14; T10 - inoculum B10.17; T11 - inoculum B10.19; and T12 - inoculum B10.20. Five replicates were used. All bacterial inoculants showed potential for phosphate solubilization and siderophore production in vitro experiments.



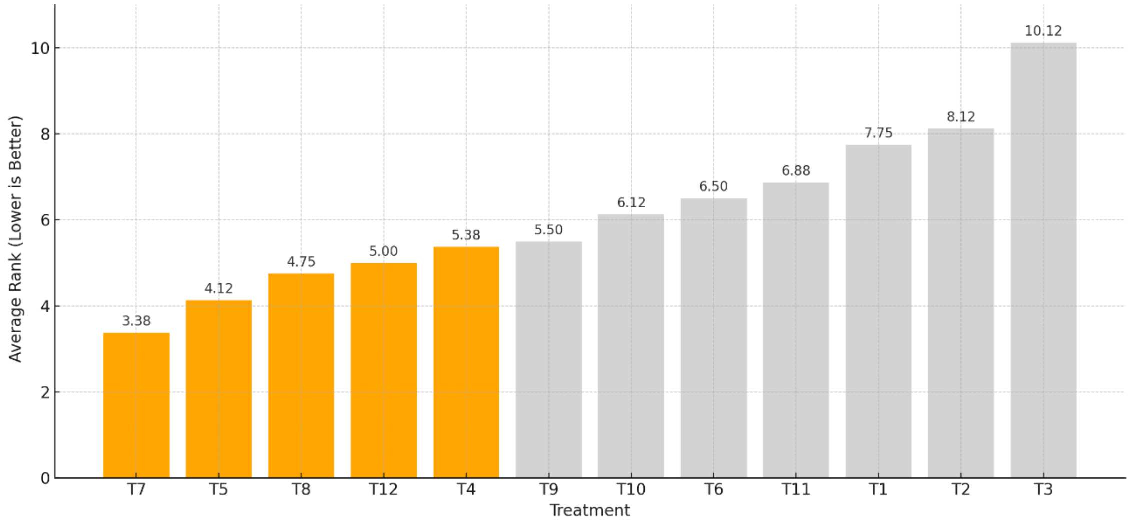
The root length (RL) variable, like ASL, showed heterogeneous behavior, also suggesting a greater difference between treatments for this variable. In the first cycle, T8, T5, and T6 had the highest values, followed by T4, which in turn had higher values than the remaining treatments. In the second cycle, T8, T12, T5, and T7 stood out, followed by T4, T11, T1, T10, and T9. The other treatments showed the worst results.

Mean Rank for Quantitative Definition of the Best Treatments

Figure 5 shows the mean rank for quantitative definition of the best treatments.

Figure 5

Mean Rank for quantitative definition of the best treatments. Lower values represent the best treatments. The top 5 treatments are shown in yellow. Legend: T1 - Conventional seedling production with P fertilization; T2 - Seedling production without P fertilization; T3 - Seedling production without P fertilization and with application of a commercial biofertilizer; T4 - inoculum B10.02; T5 - inoculum B10.05; T6 - inoculum B10.06; inoculum B10.11; T8 - inoculum B10.13; T9 - inoculum B10.14; T10 - inoculum B10.17; T11 - inoculum B10.19; and T12 - inoculum B10.20. Five replicates were used. All bacterial inoculants showed potential for phosphate solubilization and siderophore production in vitro experiments.



Mean rank was calculated by the following Equation 5:

$$Mean\ Rank = \frac{Sum\ of\ Ranks\ in\ all\ variables\ and\ cycles}{Total\ number\ of\ Ranks}$$ **Equation 5. Calculation Mean Rank (Quantitative).**

Where:

Each treatment receives a rank for each variable in each cycle;

A lower rank means better performance.

The five treatments with the lowest values are considered the most quantitative effective overall.

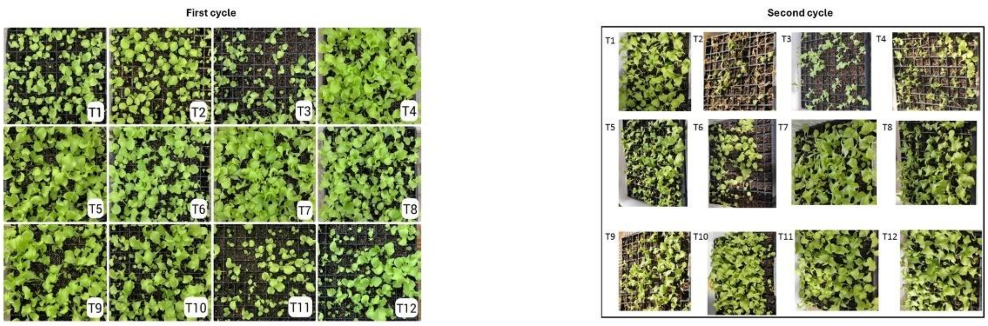
As can be seen, the average rank points to T7, T5, T8, T12, and T4 as the best treatments. However, this is a quantitative analysis and does not consider aspects such as seedlings stand uniformity and the occurrence of physiological disorders. For this reason, the next item will deal with the visual analysis of the two cycles of the experiment.

Visual Analysis Supported by Generative Artificial Intelligence (AI - LLMs)

Figure 6 shows the two photos used for human and AI evaluation for the visual ranking of the best treatments.

Figure 6

Visual comparison of lettuce seedling development in the first and second production cycles under different treatments with phosphate-solubilizing and siderophore-producing bacterial isolates.



The human evaluation, based on the same criteria used for AI-supported evaluation, Prompt Engineering, and Command Chaining, was performed by two experts in the field, both with PhDs, and returned the following result:

First cycle: T7 = T4 > T5 = T8 > T6 = T9 = T10 > T12 > T2 = T1 > T3 = T11

Second cycle: T7 = T1 = T10 = T11 = T12 > T5 = T8 > T6 > T3 > T4 > T2

Integrated ranking: T7 > T5 = T8 = T10 = T12 > T4 = T9 = T11 > T6 > T1 = T2 > T3

The evaluation carried out by the three generative AIs, using Prompt Engineering and the proposed Prompt Chaining, returned the following rankings:

ChatGPT5 – OpenAI:

First cycle: T5 = T6 = T7 > T4 > T8 > T2 > T9 > T10 > T1 > T12 > T11 > T3.

Second cycle: T7 = T8 = T12 > T5 > T6 > T1 > T10 > T11 > T9 > T4 > T3 > T2

Integrated ranking: T7 > T5 = T6 = T8 = T12 > T4 > T9 > T10 > T1 > T11 > T3 > T2

Gemini - Google

First cycle: T4 > T5 =T6 = T8 > T1 = T7 = T9 = T12 > T2 = T10 > T3 = T11

Second cycle: T7 = T11 > T1 = T5 = T6 = T9 = T10 = T12 > T8 = T2 = T3 = T4

Integrated ranking: T7 > T5 = T6 = T11 = T8 = T9 = T12 > T4 = T1 = T10 > T2 = T3

Copilot – Microsoft

First cycle: T1 = T2 > T5 > T3 > T4 > T6 > T8 > T7 > T10 > T11 > T9 > T12

Second cycle: T11 > T12 > T7 > T6 > T1 > T10 > T5 > T2 > T3 > T4 > T8 > T9

Integrated ranking: T11 > T12 > T7 > T1 = T5 = T6 > T10 > T2 > T3 > T4 > T8 > T9

ChatGPT and Gemini were the AIs that best approximated the results obtained by human observation. Microsoft Copilot AI failed badly in the ranking, indicating that there was a misunderstanding of the commands given or even a failure in the pattern recognition algorithm. Another possible interference may have been a potential increase in demand for higher-quality photos through the Microsoft Copilot component algorithm.

Table 3 shows the quantitative alignment (Spearman and MDA) and some visual observations regarding the visual analysis of the quality of the seedlings obtained with the treatments used, their ranking, and the evaluation of the results based on the use of AIs through Prompt Engineering and Prompt Chaining, proposed in comparison with the result of the integrated ranking based on human observations.

Table 3

Validation and considerations about AI-based visual ranking integration compared to human evaluation

"Evaluator"	**ρ**	**Mean Absolute Deviation (MAD)**	**Agreement Level**	**Alignment with Human Integrated Ranking**	**Validation Status**
ChatGPT x Human	**0.90**	**1.17**	**Very High**	Excellent performance, especially for top treatments (T7, T5, T8, T12)	**Validated**
Gemini x Human	**0.86**	**1.50**	**High**	Elevated consistency	**Validated**
Copilot x Human	**0.42**	**3.71**	**Low**	Elevated divergence	**Not Validated**

The use of ChatGPT5 presented the highest Spearman correlation coefficient (0.90) and lowest MDA (1.17), showing excellent compatibility with human assessment, especially for the best treatments. The use of Gemini also resulted in a high Spearman correlation coefficient (0.86) and borderline MDA. This AI showed good consistency with the results obtained by human observations. Finally, Copilot AI presented the worst results among the three AIs evaluated, with a very low correlation coefficient (0.42) and very high MDA (3.71). Thus, based on the photos presented in this work, the Generative AIs (LLMs) ChatGPT5 and Gemini were validated for use, using Prompt Engineering and Prompt Chaining. However, it is necessary for other research groups to apply these tools, as well as make possible adjustments, to infer the possibility of massive use of these digital analytical support tools.

The quantitative analysis (ANOVA + Scott-Knott + average rank) and visual analyses point to the following treatments as highlights – T7, T5 and T8, showing potential for further testing and raising the TRL/MRL maturity level, which, in this study, was conducted in a relevant environment, at the prototyping level (with conditions very similar to those used by seedling producers), is at level 6. T7 is undoubtedly the most promising treatment based on the overall results.

DISCUSSION

The use of bio-inputs in agriculture has proven to be a sustainable strategy for processes such as fertilization (Nurhayati et al., 2024**)**, biological control (Bomfim et al., 2024), and mitigation of abiotic (Junrami et al., 2022), and is of paramount importance for modern agriculture. In addition, it is in line with the 2030 Agenda and the Sustainable Development Goals (SDGs) of the United Nations (UN), as well as the Brazilian targets for achieving these SDGs 2 – Zero Hunger and Sustainable Agriculture (IPEA, 2024a), 3 – Good Health and Well-being (IPEA, 2024b), 11 – Sustainable Cities and Communities (IPEA, 2024c), and 13 – Climate Action (IPEA, 2024d). It is also in line with modern public policies at the national level, such as the National Plan for Agroecology and Organic Production, the Low Carbon Agriculture Plan (ABC+), the National Bioinputs Program, the More Sustainable Agro Brazil Program, and the National Program for Urban and Peri-Urban Agriculture.

The use of plant growth-promoting rhizobacteria in a soilless (floating) lettuce cultivation system as an alternative to mineral fertilizers resulted in increased fresh weight, number and area of leaves, dry matter of leaves, chlorophyll content, and nutrient uptake. These kinds of bacteria have agronomic multifunctionality, including phosphate solubilization and siderophore production, which would explain the positive effects of their application (Ikiz et al., 2024).

The use of co-culture between two bacterial genera isolated from soil (Pseudomonas sp. and Serratia sp.) as a biofertilizer and plant growth promoter was evaluated for onion (Allium cepa) germination and growth over two months by Blanco-Várgas et al. (2020). The authors showed that the co-culture was able to maximize phosphate solubilization through the release of phosphatase-type enzymes. After five days, 90% of the seeds had already germinated when the treatment containing the two bacterial genera at a concentration of 10^8 CFU/mL was applied. After two months, the mass obtained in the treatments that used the inoculants was more than double that produced by the control treatment.

The Scott-Knott test has been widely recommended in studies with multiple agronomic treatments due to its robustness for distinguishing means objectively, reducing the interpretive ambiguities observed in other tests such as Tukey's, for example (Malaquias, 2022). This method presents a different concept from other traditional multiple comparison tests such as Tukey, Duncan, and Dunett. The Scott-Knott test separates the means within the treatment groups into homogeneous groups, minimizing the sum of squares within groups and maximizing it between them. Thus, there is no overlap between groups (Ramalho et al., 2000).

The automatic counting of lettuce plants at different stages of development was used by Zhang & Li (2023), using specific models, demonstrating great potential for applied agronomic use. These authors consider that the development of digital tools such as computer vision and modern Artificial Intelligence are accessible means to monitor and improve agronomic performance. The use of AI techniques such as deep learning has also enabled the interpretation of environmental factors that lead to different levels of biotic and abiotic stress in lettuce leaves, reducing bias in the visual evaluation of experiments (Rathor et al., 2025). Shahriar et al. (2025) point out that Artificial Intelligence models such as large language models (LLMs) make important contributions to the agri-food sectors. Examples include the possibility of “designing” strategies for developing expert systems in agricultural and food practices with the aim of increasing the sustainability, resilience, and adaptation of production systems to environmental changes.

CONCLUSIONS

1. Quantitative analysis, using average rank, identified treatments T7, T5, T8, T12, and T4 as the most promising.

2. However, the combination of this analysis and the qualitative (visual) analysis pointed to T7, T5, and T8 as the most promising.
3. Although they presented numerically good results, T12 and T4 failed in the visual analysis, mainly in terms of stand uniformity.
4. T7 was the most promising treatment for future tests aimed at improving its maturity level.
5. T5 and T8 were less robust, more variable between the two cycles, but also promising.
6. ChatGPT5 and Gemini were the generative AIs that, based on the photos presented, Prompt Engineering, and the proposed command chain, presented results compatible with human evaluation.
7. This work is strongly aligned with modern agricultural challenges such as sustainability, resilience, and climate adaptation, as well as with the 2030 Agenda and the Brazilian legal framework that addresses sustainability in agriculture.

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